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Research Note

Influence of β-Agonists (Ractopamine HCl and Zilpaterol HCl) on Fecal Shedding of *Escherichia coli* O157:H7 in Feedlot Cattle†

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ABSTRACT

Ractopamine HCl and zilpaterol HCl, β-agonists recently approved for use in feedlot cattle to improve performance traits and carcass leanness, were examined for their effects on fecal shedding of *Escherichia coli* O157:H7 in feedlot cattle. Fecal samples ($n = 2{,}454$) were obtained from four experiments (one ractopamine HCl, three zilpaterol HCl) over the course of a 3-year period, either by rectal palpation (ractopamine HCl experiment) or from pen-floor fecal pats. Samples were cultured quantitatively and qualitatively for *E. coli* O157:H7. No significant treatment differences were detected for fecal prevalence of *E. coli* O157:H7 in the ractopamine HCl experiment. Zilpaterol HCl feeding had no effect ($P > 0.20$) on fecal shedding in the first or second experiments, with overall *E. coli* O157:H7 prevalence relatively low (<7%). In the third zilpaterol HCl experiment, the percentage of fecal samples that were *E. coli* O157:H7 positive following qualitative culture was higher ($P < 0.05$) in the zilpaterol HCl treatment (10.3%) than for the control (6.1%). The current research showed minimal effects of β-agonists on fecal shedding of *E. coli* O157:H7 and indicated that these compounds (fed immediately prior to slaughter) are not a cause for concern from a food safety standpoint.

β-Agonists (clenbuterol, cimaterol, ractopamine, zilpaterol) are organic molecules that bind to β-adrenergic receptors and repartition nutrients to increase the lean/adipose tissue ratio *(8, 9)*. Ractopamine HCl was approved for use in feedlot cattle in 2003, and in 2006 the U.S. Food and Drug Administration approved zilpaterol HCl to be fed to beef cattle in the last 20 to 40 days of the finishing period to increase rate of gain, improve feed efficiency, and increase carcass leanness *(1, 8, 9, 13)*. The ''physiological counterparts'' to synthetic β-agonists are the hormones norepinephrine and epinephrine *(7)*, reported to be involved in the quorum-sensing system that bacteria and host cells use to communicate *(12)*. This communication system has been called ''a global regulatory mechanism for basic physiological functions of *E. coli* O157:H7'' *(11)*; it regulates virulence gene expression *(12)* and stimulates the growth of intestinal populations *(5, 6)*.

Earlier research by the authors examined the effect of ractopamine HCl supplementation on fecal shedding of *Escherichia coli* O157:H7 in beef cattle, sheep, and pigs *(3, 4)*; it was our hypothesis that ractopamine HCl would act similarly to the catecholamines and thereby increase fecal shedding. However, in two experiments we observed just the opposite effect: a decrease in fecal shedding of *E. coli* O157:H7 in ractopamine HCl–treated animals. While the effects on fecal shedding (measured qualitatively as shedding or not shedding) were statistically significant, they were not 100% consistent among all pens of cattle, nor did we measure fecal shedding quantitatively. Therefore, the objectives of the current research were to examine (quantitatively and qualitatively) the effect of β-agonist (ractopamine HCl and zilpaterol HCl) supplementation on fecal shedding of *E. coli* O157:H7 in feedlot cattle.

MATERIALS AND METHODS

The sample collections described below were made from four experiments designed to evaluate the effects of various feeding strategies of Optaflexx (10% ractopamine HCl; Elanco Animal Health, Greenfield, IL) or Zilmax (4.8% zilpaterol HCl; Intervet/Schering-Plough Animal Health, De Soto, KS) on performance and carcass characteristics of feedlot cattle (results presented elsewhere). Fecal samples were collected from each of these larger experiments, with the objective of determining if supplementing feedlot cattle with either of these β-agonists influenced shedding of *E. coli* O157:H7.

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Ractopamine HCl experiment. The effect of ractopamine HCl supplementation on feedlot cattle in a commercial feedlot in the southwestern United States was examined in the fall of 2006 (October through November). Fifty-six pens of crossbred beef heifers (nine head per pen; seven pens per treatment) were used in a study using a 2 × 3 factorial design consisting of two ractopamine HCl levels (0 and 200 mg per head per day) and three ractopamine HCl feeding durations (14, 28, or 42 days). Ractopamine HCl was incorporated into the total mixed ration per manufacturer's recommendations during the final phase of the finishing period, immediately prior to slaughter. Aside from ractopamine HCl feeding and duration, all heifers were managed in a manner typical for a commercial feedlot operation in this region of the United States. One block of pens were harvested on day 1 to establish a baseline for comparison of the serial harvest data, but they were not sampled for the prevalence of *E. coli* O157:H7. Heifers were restrained in a squeeze chute, and fecal samples (approximately 50 g) were collected via rectal palpation using sterile palpation sleeves. Fecal samples were collected on day 0 (immediately prior to ractopamine HCl supplementation) and on days 14, 28, and 42 immediately prior to shipping to the slaughter facility. Fecal samples were placed on ice and shipped overnight to our laboratory in College Station, TX, for bacterial culture (described below) on the following day.

Zilpaterol HCl: experiment 1. The animals sampled in this experiment were part of a larger experiment (conducted during the summer of 2007 in the western United States) in which 320 Holstein steers were used in a feedlot trial to determine the influence and duration of zilpaterol HCl on carcass yield. Upon arrival to the feedlot, steers were weighed, eartagged, and sorted by body weight into eight groups. Within body weight groupings, steers were assigned to 10 blocks of four pens, eight steers per pen. Treatments were randomly assigned to blocks and consisted of feeding zilpaterol HCl for 0 (control), 20, 25, or 30 days. All cattle were fed the control diet for a 9-day acclimation period prior to initiation of the experiment. Zilpaterol HCl was fed at 7.56 g/ton of the total mixed ration (100% dry matter basis). Following a 3-day withdrawal period, 20 blocks of cattle were shipped to slaughter; the remaining 5 blocks were shipped the following day. Fresh fecal samples (five per pen) were collected from the pen floor using sterile palpation sleeves at the time of shipment to the slaughter facility. One pen per treatment was used for animals not performing well on the study (for various health and other issues) and was therefore excluded from sampling ($n = 180$ fecal samples collected). Samples were shipped as above for processing.

Zilpaterol HCl: experiment 2. Fecal samples were collected from two groups of crossbred beef steers located on a commercial feedlot in the southwestern United States. Each group of cattle had two staggered slaughter dates (January to April 2007). Fecal samples (collected as above) were obtained from each group of cattle on the later slaughter date (February and April) based on the expected low seasonal prevalence of *E. coli* O157:H7. Cattle were housed in drylot pens (approximately 100 head per pen) and subjected to management and feeding as normal for this region of the United States. Within each group of cattle, zilpaterol HCl (7.56 g of zilpaterol HCl per ton of total mixed ration [100% dry matter basis]) was fed for 0 (control), 20, 30, or 40 days prior to slaughter. A 3-day withdrawal period immediately preceded slaughter. Three pens from each treatment (feeding duration), representing both groups of cattle, were sampled (30 samples per pen; 360 samples per group; $n = 720$ total). Treatments were the same for each group, but due to the different collection times, data were analyzed and are presented by group (date of collection).

Zilpaterol HCl: experiment 3. Crossbred beef heifers located on a commercial feedlot in the southwestern United States were fed 0 (control) or 7.56 g of zilpaterol HCl per ton of total mixed ration (100% dry matter basis) for 20 days immediately prior to slaughter. There was a 3-day withdrawal period immediately prior to slaughter. Animals were housed in drylot pens (approximately 95 head per pen) and managed as typical for a feedlot in this region of the United States. Cattle harvest dates were staggered; therefore, two fecal sample collections were made in June and July 2008, within 4 days of slaughter. Thirty fecal samples per pen (nine pens per treatment) were collected prior to each harvest date, for a total of 360 samples per date or 720 total samples. Fecal samples (approximately 50 g) were collected from freshly voided fecal pats, placed in sterile specimen cups, and shipped to our laboratory as described previously.

Bacterial culture, isolation, and enumeration methods. All fecal samples were processed the day following collection for qualitative and quantitative analysis of *E. coli* O157:H7 as described previously *(10)* and modified *(2)*. For the ractopamine HCl study only, all fecal samples were kept refrigerated and all immunomagnetic separation–positive samples were further examined for quantitative analysis of *E. coli* O157:H7. One gram of feces was serially diluted in phosphate-buffered saline, and 0.1-ml aliquots were plated and confirmed as *E. coli* O157:H7 as above. Enumeration of *E. coli* O157:H7 for the remainder of the studies was performed as referenced above *(2, 10)*.

Statistical analysis. Data were analyzed using SAS version 8.02 (SAS Institute, Inc., Cary, NC). The percentage of cattle shedding *E. coli* O157:H7 and the percentage of pens positive (pen with at least one animal shedding) were subjected to chi-square analysis using the PROC FREQ procedure. The concentration of *E. coli* O157:H7 [CFU (log)/g of feces] was subjected to analysis of variance appropriate for a completely randomized design. A value of 1.0 was assigned to all culture-negative animals for analysis of the quantitative shedding data. When appropriate, data were analyzed by duration of β-agonist feeding. Differences in means were considered significant at $P < 0.05$.

RESULTS

Ractopamine HCl experiment. The effect of ractopamine HCl supplementation on the percentage of heifers shedding *E. coli* O157:H7 is presented in Table 1. Concentrations of *E. coli* O157:H7 in all samples, with one exception, were below our detection limit of 2.3 CFU (log)/g of feces, and therefore all data presented are from the qualitative assay. No treatment differences ($P > 0.20$) were observed prior to (day 0) or following 28- or 42-day ractopamine HCl supplementation. Ractopamine HCl supplementation for 14 days tended to decrease ($P < 0.11$) the percentage of heifers shedding *E. coli* O157:H7 compared with control animals. When expressed as percent change in prevalence from day 0 to day 14, shedding in the ractopamine HCl treatment was unchanged, whereas in the control animals shedding increased nearly 15% (Table 1). Pen status (percentage of pens with at least one heifer shedding *E. coli* O157:H7) is presented in Table 2. No significant differences were observed except on day 0 of the

TABLE 1. *Effect of ractopamine HCl on the percentage of heifers shedding* E. coli *O157:H7 in their feces*

	Treatment[a]:			
	Control		Ractopamine HCl[b]	
Feeding duration	*n*	%	*n*	%
14 days				
Day 0	7/69	10.1	10/71	14.1
Day 14	18/72	25	10/70	14.3
% change		+14.9		+0.2
28 days				
Day 0	11/71	15.5	10/71	14.1
Day 28	15/72	20.8	12/71	16.9
% change		+5.3		+2.8
42 days				
Day 0	13/70	18.6	7/62	11.3
Day 42	10/72	13.9	6/63	9.5
% change		−4.7		−1.8

[a] Data (number and percentage of positive samples) are presented by feeding duration. Samples were collected 1 day prior to ractopamine HCl supplementation (day 0) and again following 14, 28, or 42 days of ractopamine HCl feeding.
[b] Supplementation (200 mg per head per day) fed in a total mixed ration to feedlot cattle.

TABLE 2. *Effect of ractopamine HCl on the percentage of* E. coli *O157:H7–positive pens (at least one heifer shedding* E. coli *O157:H7)*

	Treatment[a]:			
	Control		Ractopamine HCl[b]	
Feeding duration	*n*	%	*n*	%
14 days				
Day 0	4/8	50	6/8	75
Day 14	7/8	87.5	7/8	87.5
% change		+27.5		+12.5
28 days				
Day 0	6/8	75	6/8	75
Day 28	7/8	87.5	5/8	62.5
% change		+12.5		−12.5
42 days				
Day 0	8/8	100 A[c]	3/7	42.9 B
Day 42	4/8	50	5/7	71.4
% change		−50		+28.5

[a] Data (number and percentage of positive pens) are presented by feeding duration and across days. Samples were collected 1 day prior to ractopamine HCl supplementation (day 0) and again following 14, 28, or 42 days of ractopamine HCl feeding.
[b] Supplementation (200 mg per head per day) fed in a total mixed ration to feedlot cattle.
[c] Row percentages with different letters differ significantly ($P < 0.05$).

42-day supplementation period, when fewer ($P = 0.01$) ractopamine HCl pens were positive compared with control pens.

Zilpaterol HCl: experiment 1. Overall, the percentage of fecal samples positive for *E. coli* O157:H7 was 6.1% (11 of 180). Only two samples were positive via direct plating, one each in the control and the 25-day zilpaterol HCl treatment. No differences ($P > 0.10$) were observed in fecal shedding when analyzed by feeding duration (4.4, 6.7, 8.9, and 4.4% *E. coli* O157:H7 positive for control, 20-, 25-, and 30-day zilpaterol HCl treatments, respectively). The percentage of *E. coli* O157:H7–positive pens was not different among treatments, averaging 22.2, 33.3, 33.3, and 22.2% positive for control, 20-, 25-, and 30-day zilpaterol HCl treatments, respectively (data not shown).

Zilpaterol HCl: experiment 2. The prevalence of fecal shedding of *E. coli* O157:H7 was very low in the first group of cattle sampled (data not shown). All samples were culture negative following direct plating, and only three samples (one each in control, 20-, and 40-day zilpaterol HCl treatments) were positive following enrichment and immunomagnetic separation culture techniques. The collection made from the second group of cattle later in the year produced more *E. coli* O157:H7–positive samples. One sample (control treatment) was positive following direct plating, whereas qualitative determination yielded 5.6, 5.6, 8.9, and 0% *E. coli* O157:H7–positive samples in control, 20-, 30-, and 40-day zilpaterol HCl treatments, respectively. No significant treatment differences were observed among treatments when examined by zilpaterol HCl feeding duration (data not shown).

Zilpaterol HCl: experiment 3. Results for fecal shedding of *E. coli* O157:H7 are presented by collection (June and July) and across collection times in Table 3. Three (1.7%) fecal samples from the control and four (2.2%) from the zilpaterol HCl treatments had quantifiable concentrations of *E. coli* O157:H7 in June and one each in the July collection, with no significant treatment differences observed. The percentage of fecal samples that were *E. coli* O157:H7 positive following qualitative culture in the zilpaterol HCl treatment was higher ($P < 0.05$) during the June collection, not different ($P > 0.10$) in July, and higher ($P < 0.05$) for zilpaterol HCl (10.3%) than for control (6.1%) treatments when examined across collections. The percentage of *E. coli* O157:H7–positive pens was not different ($P > 0.10$) among treatments at either collection time or when combined across times (data not shown).

DISCUSSION

Results of the ractopamine experiment failed to confirm previous findings by Edrington and coworkers *(3)* that ractopamine HCl supplementation appeared to decrease the shedding of *E. coli* O157:H7 in feedlot cattle. A slight, nonsignificant decrease in shedding was observed following the 14-day supplementation period but not after 28 or 42 days of ractopamine HCl supplementation. Reasons for the

TABLE 3. *Prevalence and concentration of* E. coli *O157:H7 in fecal samples collected from two groups of feedlot cattle in June or July 2008 (zilpaterol HCl experiment 3)*

		Treatment[b]:					
		Control			Zilpaterol HCl[c]		
Date	Method[a]	*n*	%	CFU	*n*	%	CFU
June	DIR	3/180	1.7	3.9	4/180	2.2	4.8
	ENR	8/180	4.4 A[d]		19/180	10.6 B	
July	DIR	1/180	0.6	6.4	1/180	0.6	4
	ENR	14/180	7.8		18/180	10	
Overall	DIR	4/360	1.1	5.2	5/360	1.4	4.4
	ENR	22/360	6.1 A		37/360	10.3 B	

[a] Fecal samples were plated directly to quantify bacterial populations (DIR) or enriched prior to plating for qualitative analysis (ENR).

[b] Prevalence values are number and percent positive. Concentration values are CFU (log)/g of feces and represent the average of only those samples with quantifiable concentrations. For statistical analysis, all negative samples were assigned a value of 1.0.

[c] Feedlot cattle were fed 7.56 g of zilpaterol HCl per ton of total mixed ration for 20 days prior to slaughter.

[d] Row percentages with different letters differ significantly ($P < 0.05$).

differences between this study and previous ractopamine research *(3)* are unknown. Supplementation rates in the two studies were identical, and both used beef heifers. As the effects observed previously were not dramatic, it is possible that slight differences in breed, management techniques, rainfall, pen conditions, or other factors may have contributed to the lack of response to ractopamine supplementation in the current study. Because of the sporadic nature and seasonality of *E. coli* O157:H7 shedding in ruminants, detecting statistically significant differences in naturally colonized animals can be difficult. In the current research, the percentage of animals shedding *E. coli* O157:H7 was reasonably consistent among collections, averaging approximately 15% across all sampling times; therefore, we feel there were sufficient numbers of positive animals with minimal shedding fluctuations, allowing for a reasonable test of our objectives and hypothesis. However, the prevalence was lower than in previous research *(3),* which may have influenced the results. Although we had hoped that quantitative determination of *E. coli* O157:H7 populations, not conducted in previous research, would provide for a better assessment of the effect of ractopamine on fecal shedding, concentrations were below our detection limits and failed to yield any beneficial data.

Results from the three zilpaterol HCl experiments similarly failed to provide any strong evidence of a zilpaterol and *E. coli* O157:H7 relationship. The prevalence of *E. coli* O157:H7 was likely too low in the first two experiments to detect any treatment differences; results from the third experiment appear to support our original hypothesis, but not our first results *(3)*. Originally we had hypothesized that if β-agonists had any effect on *E. coli* O157:H7, it would be the same as that reported for the catecholamines and would increase prevalence of fecal shedding. However, our results were just the opposite, with ractopamine HCl decreasing *E. coli* O157:H7 prevalence in feedlot cattle *(3)*. In the current research (zilpaterol HCl experiment 3), we observed an increase in *E. coli* O157:H7 prevalence, but not concentration, in zilpaterol HCl–supplemented feedlot cattle compared with controls. Reasons for these differences are unknown; they may simply be a function of different responses to the two β-agonists.

The effects of β-agonists on fecal shedding of the foodborne pathogen *E. coli* O157:H7 appear to be mild at best and may better be described as negligible. While a β-agonist–related reduction in fecal shedding of these pathogens would certainly be a desired result since these compounds are fed immediately prior to slaughter to improve feedlot performance and carcass leanness, a lack of effect on pathogenic bacteria (contrary to our original hypothesis) is also good news.

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